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THE ACTION OF SAPONIN AND ITS NON-HEMOLYTIC MODIFICATION ON GROWTH[1]

JOSEPH M. BUTROS

Biology Department, American University of Beirut, Beirut, Lebanese Republic

The hemolytic activity of saponins has been extensively studied and has stimulated investigations related to other properties of these compounds. A series of studies on seed germination and plant growth are reported by Balansard and Pellissier (1943; 1944; 1945) and indicate a growth-promoting activity of saponin in very dilute solutions (10 to 100 ppm.). Such solutions produce considerable proliferation forming tumor-like concrescences in ivy shoots (1943). Seedlings from treated cereal seeds grow faster and become larger than their controls (1944). Similarly the germination of tomato seeds was accelerated (1945). Dilute saponins accelerate water absorption of moistened seeds and speed up germination of corn (Balansard, Pellissier and Conil, 1946).

On the other hand, the inhibitory action of saponins in higher concentration has been reported by some authors. Amerio and Dalla (1942) found that the roots of *Allium cepa* and coleoptiles of oat were irreversibly but non-specifically damaged. Von Euler (1946) found that seedlings of *Lepidium sativum* and *Hordeum vulgare* grew to only one-third to one-tenth of the lengths of the controls. Inhibitory action is also reported in animals: heart and lung tissue of hen embryo (Hideo, 1928) and Jensen sarcoma (Frey, 1938).

Two years ago the author described the cytolytic effects of 0.5 per cent Merck saponin on rat tissue and tumors (Butros, 1948). The Feulgen stain showed degenerative changes in the nucleus. This suggested a nucleo-toxic action and the necessity of a study of the effects of lower concentrations, especially on growing tissues. As onions have often been used in the study of mitosis and growth, they were here also employed in the greater part of these experiments.

Another aspect of saponin action was investigated also, *i.e.*, the possibility that its nucleo-toxic action is independent and separate from its hemolytic action. Hemolysis can be inhibited by a combination of saponin with cholesterol (as will be discussed further on). The nuclear damage might be another matter, and might depend on the action of saponin on some constituent other than those involved in hemolysis. If so, its action might show itself by interfering with mitosis and growth even if it has lost its hemolytic power. After sufficient indications of inhibitory action of both saponin and its "cholesteride" were evident by trials with onion roots,

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the author started investigations on animals. The toxicity of the "saponin cholesteride" was much less than saponin of the same concentration and so it was felt safe enough to test it on tadpole development. Experiments with a non-hemolytic saponin-eosin preparation are also described.

The Saponins Used

Two saponins have been used in this work: Merck's (white pure), and Baker's (yellow). Solutions were made in tap water for the onion roots, in distilled water for the others.

The hemolytic strength of these two saponins was not the same. Hemolytic tests in this paper refer to the standard method of Ponder (1948). The time of complete hemolysis is the one considered except in some cases, especially of "saponin cholesteride," where an approximation of the percentage of hemolyzed r.b.c. by the microscope was employed.

Baker saponin in 1/1000 dilution resulted in complete hemolysis in three minutes. A 1/10,000 solution needed 18 hours to complete hemolysis, but a solution of 1/15,000 brought about only 75 per cent hemolysis in the same length of time. Merck saponin solutions of 1/50,000 show complete hemolysis in 18 hours; 1/100,000 show 50 per cent hemolysis in 18 hours and 1/200,000 show 25 per cent hemolysis in the same time.

Action of Saponin on Onion Root Growth

Onions (*Allium cepa*) were sprouted in water before placing them in the saponin solutions. Whole and half onions were used, the sister halves of the treated being used as controls in water. Measurements of root lengths were made and sample roots were fixed for cytologic preparations. Roots that had stopped growth in saponin were returned to water to determine their ability to resume growth. Very dilute solutions of saponins were included in this study to compare with the work of Balansard and Pellissier referred to in the introduction.

Inhibitory Concentrations

An arrest of root lengthening was definitely established within one or two days, depending on individual susceptibility, after placement in 0.05 per cent to 0.1 per cent Merck saponin and 0.25 per cent to 0.3 per cent Baker saponin. If roots were returned to water at this point, most of them were able to resume growth, but if left more than four days in the saponins they could not. The new growth was easily distinguished by its fresh, white texture as compared with the brownish treated tips. There was often a knot-like hypertrophy at the junction between the area grown in saponin and that grown in water. Curvature of the roots was noted in saponin but not in the controls. If left in the solutions over a week, the arrested roots developed secondary roots that came to a standstill in a couple of days. New roots from the periphery of the onion sometimes developed (Fig. 1).

From the study of sections taken at intervals of 12 hours for five days in saponin, it became clear that the mitotic process was inhibited completely by the second day, when no mitotic process was found. All cells were in the resting stage or in what appeared as very early prophase (Fig. 2). The chromatin particles were small,

thin rods. No disturbances or aberrations were noticed in the mitotic figures of the first day except that they were somewhat less numerous than the control. The region of elongation was not modified. Comparisons between microscopic and length studies were in agreement; roots that resumed lengthening after return to water showed recurrence of normal mitoses as abundant as the controls.

FIGURE 1. Onion that was replaced in water after treatment with 0.1 per cent Merck saponin, showing arrested roots with knotty beaded tips and secondary growth.

Lethal Concentrations

If roots were left long enough (4 to 5 days) in an inhibitory concentration, or for two days in a higher concentration, cytolytic degeneration occurred, ending in death of the desiccated roots. The nuclei became first pycnotic, then shrunken, pointed and eccentric. The cytoplasm gradually vacuolated out. Cells at the root cap were also displaced and disintegrated.

Sub-Inhibitory Concentrations

Experiments were done as described above: half onions were placed in the saponin solutions and their control halves in water. The length that represented

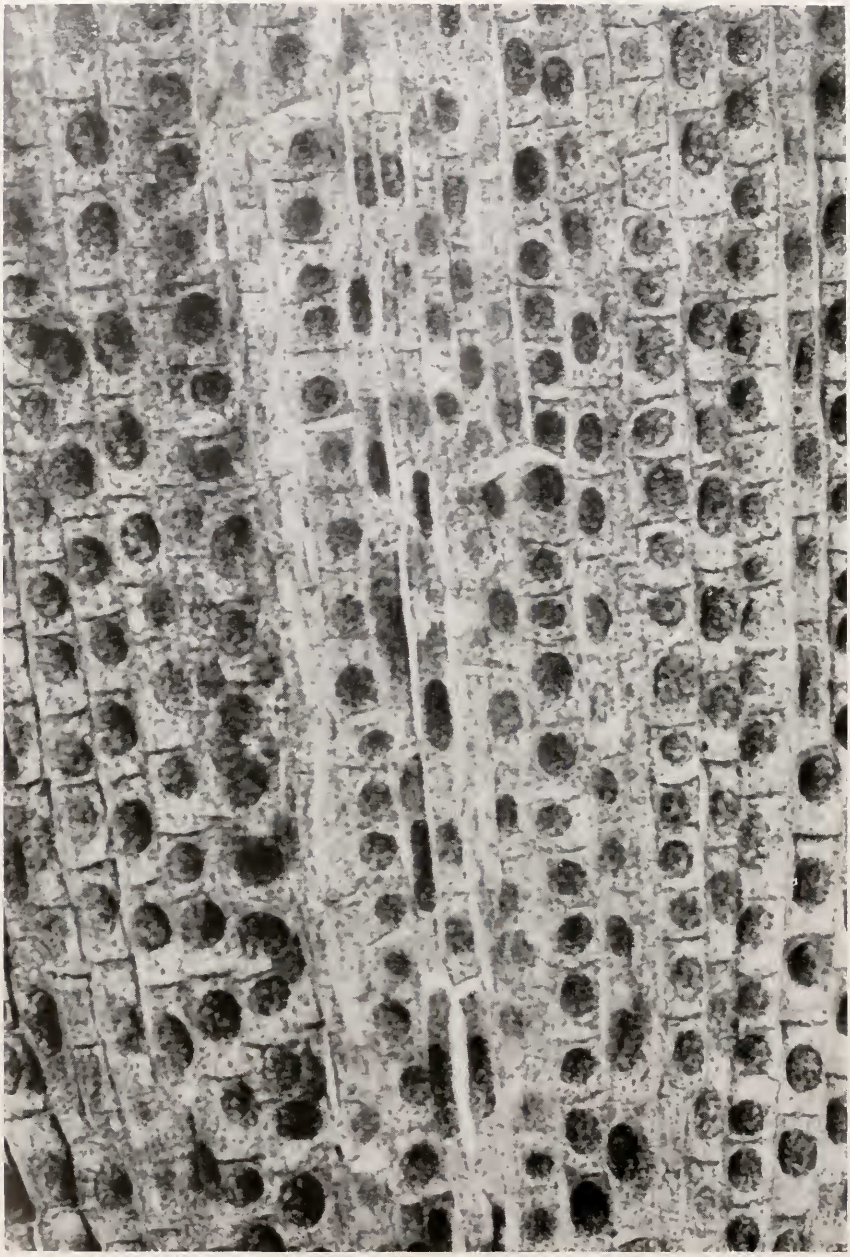

FIGURE 2. Section of onion root showing inhibition of mitosis in 0.1 per cent Merck saponin; fixed in Bouin's and stained with hematoxylin and eosin. × 400.

the majority of the roots in each specimen was tabulated daily, from which increments were calculated. The concentrations used were:

Merck: 1/5000; 1/10,000; 1/100,000; 1/500,000
Baker: 1/1000; 1/2000; 1/10,000; 1/500,000; 1/1,000,000; 1/2,000,000

As a summary, Table I gives the length measurements and compares the growth rates of the various concentrations of Baker saponin.

For a check on the deviation between normal onion halves an experiment was run with onion halves in two sets of vessels, both containing water. The average difference in length between the two sets at the end of 12 days was 0.34 cm. This value was used in evaluating the significance of experimental results.

TABLE I

Growth of onion roots in various concentrations of Baker saponin

No. of Days in Solution	Concentration	Length in Saponin	Length in Water	Increase in Saponin	Increase in Water	Growth Index*
7	1/2,000,000	9.9 cm.	8.9 cm.	8.9 cm.	7.9 cm.	1.1
10	1/500,000	11 cm.	9.3 cm.	10 cm.	8.3 cm.	1.2
6	1/10,000	8.2 cm.	9.4 cm.	7.2 cm.	8.4 cm.	0.9
8	1/2000	7.1 cm.	9.4 cm.	6.1 cm.	8.4 cm.	0.71
5	1/1000	5.1 cm.	8.4 cm.	4.1 cm.	7.4 cm.	0.53

* Increment per day in saponin divided by increment per day in water.

On examining Table I it will be seen that the very dilute concentrations of Baker saponin may be considered to favor growth. This agrees with the findings of Balansard and Pellissier referred to above. The lowest dilutions of Merck saponin used in these experiments did not show this growth-promoting power. As Merck saponin is stronger, they really compare to higher concentrations in terms of Baker saponin, which are also non-growth promoting.

Microscopic examination of root sections treated with the sub-inhibitory solutions of saponin showed apparently normal mitotic figures and healthy cells. The sub-inhibitory dose, however, is capable of producing inhibition of mitosis and cytolysis of cells if the roots were left long enough in the solution. After 6 days in 1/10,000 Merck saponin, the cells were vacuolated and no mitotic figures were discernible.

The Respiration of Onion Roots in Saponin

Trials were made to find out if saponin suppressed the respiration of onion roots. If it did, its inhibitory action could have been due to, or at least related to such action. The indicator bromthymol blue was used in most cases. This was very reliable as shown by repeated trials with growing roots, roots killed in alcohol, and roots immersed in KCN. It was found that in 0.3 per cent Baker saponin (which is sufficient to stop mitosis in two days), the roots were able to decolorize bromthymol blue, indicating production of an acid; pH measurements of the solutions containing the onions served as a further check on the production of carbon dioxide. The pH of the saponin solution in which onions grew was 6.4. Its control with no onion was

7.4, and a second control with a dead onion was 7.6. Further, distilled water containing respiring onions had a pH of 5.4 on the second day; its control with no onion was 6.0. These results show that onion roots continue to respire in saponin while their mitotic process is gradually coming to a standstill.

Preparation of Non-Hemolytic "Saponin Cholesterides"

In the extensive article on saponins by Kobert-Rostock (1924), it is explained that all saponins lose their hemolytic activity when their solutions are warmed with cholesterol. Windaus (1909) made a study of this reaction by using a steroid saponin, digitonin, which, although different in structure from the ordinary saponins, shares in their hemolytic activity. He made a digitonin cholesteride compound by precipitating it from an alcoholic solution of the two compounds. The cholesteride is not separated into its constituents by ether but is separated by boiling xylene. Using ordinary saponin, Meyerstein (1910) rendered it non-hemolytic by shaking it with cholesterol in normal saline. He stated that it is not of great importance whether these two compounds were held up by a genuine chemical combination or only by adsorption. His preparations were freshly made when needed.

The author used a modification of Meyerstein's method, which gave a stable non-hemolytic suspension that could stand over two months without splitting back into saponin and cholesterol. The method depended upon the use of a homogenizer [2] which ground the cholesterol into very fine particles. This was run into hot saponin solution and the reaction (or adsorption) took place instantaneously, as shown by the loss of frothing and loss of hemolytic action. Comparison with hemolytic tests made on saponin was very helpful in determining approximately the amount, if any, of free saponin in the suspension, and so indicating the possibility that the biological action might be due to free saponin.

A phosphate buffer was sometimes added to the "saponin cholesteride" when it was used with tadpoles. This seemed to favor the adsorption of the two components and make the compound more stable. The proportions of the components used were:

Baker saponin 3 gm. per liter; cholesterol 1.5 gm. per liter
Merck saponin 3 gm. per liter; cholesterol 2.5 gm. per liter

Saponin did not become non-hemolytic except when there was an excess of cholesterol. The suspension remained milky and non-hemolytic for over two months.

Properties of the "Saponin Cholesteride"

If the suspension is prepared successfully, it does not froth, is milky with the larger particles settling down, but it does not clarify even within three months. Merck's preparation is white, and Baker's light brown. Sometimes, for unknown reasons, it was not possible to make the suspension as there was a flocculation. The clear fluid, however, of the flocculated preparation was non-hemolytic and had the same inhibitory property as the milky suspension.

The clear filtrate of the "saponin cholesteride" remained non-hemolytic for a few days during which it was inhibitory to onion roots like the mother compound.

[2] The author is deeply indebted to Messrs. Awad and Baramki of the Chemistry Department for their help in this matter.

The color of the filtrate of the Baker preparation was pinkish, whereas the color of solutions of this saponin was yellow and never pink, no matter how dilute. The clear filtrate does not froth also, another indication that it does not contain free saponin. It could have been a saponin-cholesterol complex.

Extraction with ether of the Merck cholesteride until all excess cholesterol was removed gave a milky suspension with no microscopically visible crystals of cholesterol. This extract was non-hemolytic also (after evaporation of the hemolytic ether), but it became hemolytic on standing a few days, possibly due to the lack of excess cholesterol.

Thus it seems that the "saponin cholesteride" prepared in this manner was of variable and uncertain composition, and deserves a detailed study.

Action of "Saponin Cholesteride" on Onion Roots

Whole and half onions were placed in the suspension; controls were in water and in suspended cholesterol. In all cases, concentrations refer to the amount of saponin in the suspension.

Results in Baker Saponin

A 1.8 per cent suspension checks the growth in length of the roots in two days. The tips have the knotty appearance which was noted in saponin, and they curve upwards. A 0.9 per cent suspension was also able to check the growth of the roots within two days. Microscopic examination revealed the absence of mitotic figures, all cells being in the resting stage. Signs of beginning degeneration were apparent on the third day, indicated by a very little vacuolation of the cytoplasm and slight shrinkage of nuclei. A few roots with more resistance showed four or five mitotic figures per field ($\times$ 250). In the 0.3 per cent suspension, mitotic figures were found in the slides but about 10 per cent less than in the control.

Results with 0.3 Per Cent Merck "Saponin Cholesteride"

This concentration was insufficient to stop growth and mitosis completely. However, there was a 50 per cent reduction in the number of mitoses as compared with water control, and with dilute saponin control. The rate of lengthening dropped to 30 per cent. The roots were thicker than usual and the microscopic view indicated hypertrophy of the cells. It can be assumed from trials with Baker saponin that if the concentration were raised, complete arrest would result.

Proof That the Action is Not Due to Free Saponin

The hemolytic power of various concentrations of the "saponin cholesteride" was determined to help in approximating the amount, if any, of free saponin in the cholesteride. It should be remembered that complete hemolysis occurs within 18 hours in a concentration of 1/10,000 of Baker saponin but not if the concentration is 1/15,000 or 1 20,000. As the concentration of saponin required to stop mitosis is 0.3 per cent, which causes hemolysis in a few minutes, and as the "saponin cholesteride" did not show complete hemolysis in a much longer period (24 hours), there is no possibility of its action being due to saponin. Similarly, in the case of Merck saponin, there is immediate complete hemolysis in up to one per cent. The mitotic inhibitory dose is within this range, and as there was no complete hemolysis of the

"cholesteride" overnight, it is out of the question that the inhibition (partial in this case) was due to free saponin.

Daily hemolytic tests were made from samples of "saponin cholesteride" in which onions were growing, to eliminate the possibility that any saponin was splitting from the cholesteride as a result of the action of some agent from the onion. No splitting took place when only the roots were immersed in the suspension.

Filtered Merck "saponin cholesteride" (0.3 per cent) did not cause complete hemolysis in 24 hours. It arrested the growth of onion roots after a growth of ½ cm. during the first day.

Controls

Onions were grown in cholesterol suspension made by the homogenizer of the same strength as the 0.3 per cent cholesteride (which is partially inhibitory). The rate of growth in length was as follows:

In water	1 cm. per day
In cholesterol	0.8 cm. per day
In "saponin cholesteride"	0.4 cm. per day

In another experiment, corresponding half onions were placed in cholesterol and water respectively. The cholesterol in this case was ground in a mortar and then shaken several hours by a shaking machine to the same strength as the "saponin cholesteride." There was no apparent difference between the length of roots in cholesterol with those of the water.

Curvature of Onion Roots

Root curvature in saponin and its "cholesteride" suggested interference with auxins, although the latter are concerned with root sprouting rather than with root growth (Went and Thimann, 1937). If the "cholesteride" interferes with auxin action, then it might prevent the sprouting of roots, so onions were placed in these solutions. In both saponin and the "cholesteride," the onion roots appeared and grew for two to three days. However, they stopped at the length of 1.5 cm., as they usually do if placed after sprouting. It does not seem that curvature in these solutions had a relation to auxin action.

Toxicity of "Saponin Cholesteride" to Animals

A comparison of the toxicity of saponin and its "cholesteride" of the same concentration was made by noting their killing time on certain animals. In general the saponin toxicity was greatly decreased. For example, paramecium is cytolyzed instantaneously in 0.45 per cent Baker saponin, whereas it continued to move about for 5 hours in double that concentration of "cholesteride."

Table II compares the duration of life in Baker saponin and its "cholesteride" for the fresh water snail *Melanopsis bovieri* and planaria.

Toxicity studies were also made on tadpoles, and it was clear that the toxicity was greatly reduced when compared to that of pure saponin. It took 12 hours to kill tadpoles in 0.9 per cent "saponin cholesteride," whereas saponin of that concentration killed them in a few minutes. The "cholesteride" was non-hemolytic and this property was tested daily and in particular during the interval that the tadpoles were

in the suspension. This is significant as it points to a toxic property independent and separate from the known hemolytic property.

Filtered Merck "cholesteride" (0.2 per cent) kills tadpoles in 5 hours and shows complete hemolysis in less than 20 hours at 37° C. At room temperature it does not show complete hemolysis in that time, however. The ether-extracted

TABLE II

Duration of life in Baker saponin of Planaria and snails

Concentration	Saponin		Saponin Cholesteride	
	Planaria	Snail	Planaria	Snail
0.9%	5 minutes	3.5 minutes	3–5 hours	1 hour
0.45%	4 minutes	4 minutes	10 hours	8 hours
0.09%	30 minutes	5 minutes	24 hours	20 hours
0.05%	60 minutes	6 minutes	40 hours	30 hours

"cholesteride," which is free of cholesterol particles, also kills the tadpoles in 5 hours and produces 50 per cent hemolysis in 20 hours. If we assume a very low content of free saponin, such as 1/200,000, which has the same hemolytic power as the above suspensions, we still cannot explain the killing action of these suspensions, because the tadpoles can tolerate this much saponin overnight.

Cholesterol Control

A suspension of cholesterol in water kills the tadpoles within 5 hours, possibly by the accumulation of crystals in their gills. If the suspension is filtered, it becomes harmless.

Cytolysis of Mammalian Tissues

Slices of liver, kidney and intestine of the rat were placed aseptically in 0.3 per cent Merck "cholesteride" and similarly in 0.3 per cent saponin for 7 hours in the ice box. Microscopic study of prepared sections revealed almost equivalent degree of cytolysis in both solutions. The liver was more damaged by saponin than by its "cholesteride," as judged by the dissolution of chromatin resulting in a cloudy homogeneous nucleus. The shrinkage of the liver tissue was enormous in both cases; many wide, long spaces were observed among the liver cords.

The intestinal epithelium was greatly dehydrated and damaged. The cells were almost devoid of cytoplasm. The nuclei were shriveled points. The effect of the "cholesteride" in this case was more striking than that of saponin.

The kidney nuclei were pycnotic in both solutions. The resistance of this organ was greater than that of the liver and the intestine.

Hemolytic tests were performed at the beginning and at the end of the immersion period of the tissue slices, and the "saponin cholesteride" was found nonhemolytic in 20 hours. It seems, then, that "saponin cholesteride" is as effective as saponin in causing the cytolysis of tissue cells.

Action of "Saponin Cholesteride" on Tadpole Development

It was mentioned under the properties of this compound that its toxicity as compared to saponin was greatly decreased, to such an extent that tadpoles could stand 0.9 per cent Baker "saponin cholesteride" for several hours with no serious damage. The tadpoles became quite irritable at first, moving rapidly in all directions, then they quieted down as if anaesthetized and remained so to the end of the period of immersion (1 to 2 hours). During this treatment their heart rate drops down to 30 beats a minute, as compared to 100 beats of the normal (chloretone anaesthetized) tadpoles. When washed and returned to water they remain "intoxicated" for an hour or so before they regain their normal activities and normal heart rate.

Tadpoles of various stages as well as cleaving eggs were treated by short immersions in "saponin cholesteride" and the effect on development was noted by comparing with the controls. All tadpoles were kept in finger bowls and fed boiled spinach leaves.

Trials with Cleavage Stages

Female frogs were induced to ovulate by injecting several pituitary glands in the abdomen of each. They were stripped into a suspension of minced testes. When all ova were in the two-cell stage, they were divided into four groups and placed in the following solutions:

1. 0.15 per cent Baker saponin
2. 0.9 per cent Baker "saponin cholesteride"
3. 0.0025 per cent KCN control
4. Water control

Within 5 hours, eggs in the four groups cleaved to the 16-cell condition. By 20 hours the eggs in the three agents reached the blastula or Stage 8,[3] while those in water reached late blastula or Stage 9. After 64 hours, both saponin and "saponin cholesteride" were still in blastula, and did not resume development after return to water. KCN treated eggs were in Stage 12 and water group in Stage 14. It was apparent that there was a generalized poisoning, due to continuous immersion in the solution, in the saponin and its "cholesteride."

Unhatched Batch (Stage 17)

These were immersed 2 hours daily in 0.9 per cent suspension for a period of two weeks. On the second day after treatment, 11 tadpoles hatched from the water control but none from the "cholesteride" group. On the ninth day, inspection of the organisms showed that the treated groups were smaller. They still had their suckers, whereas the controls had lost them. Treatment stopped after two weeks. On the fiftieth day from the beginning of the experiment, the treated animals averaged 2.8 cm. in length; their hind limbs were in Stage 7. The controls were 3.5 cm. and their limbs in Stage 11 (Fig. 3).

Hatched Tadpoles (Stage 19)

On the fifth day, it was noticed that the water control group were ahead of the "cholesteride" group in having their mouths open and their eyes more pigmented.

[3] In this paper, stage numbers are according to Rugh, 1948.

On the twenty-sixth day the tadpoles in water averaged 2×0.5 cm., while those in "cholesteride" were 1.4×0.3 cm.

Tadpoles of Stage 21

Daily immersion in 0.9 per cent "saponin cholesteride" for 15 days, after which they were returned to water. On the fiftieth day, the water controls had complete

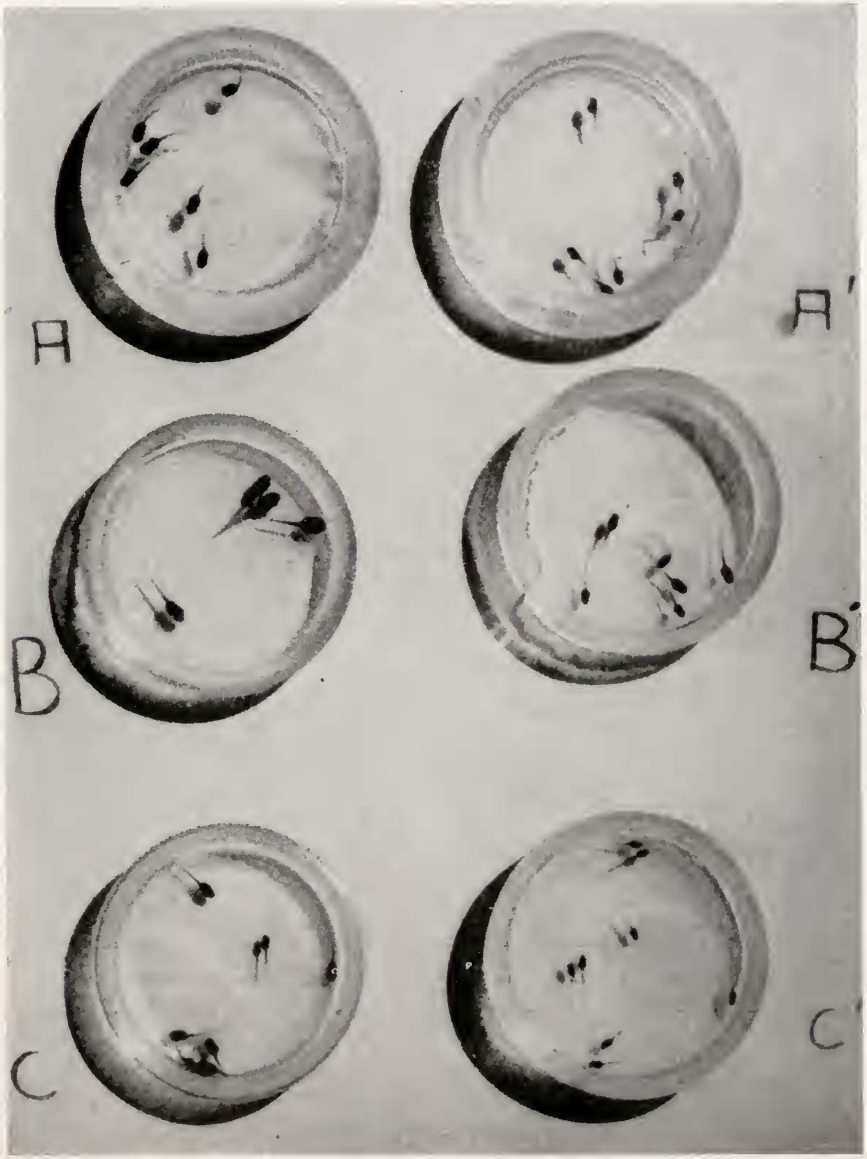

FIGURE 3. A', B', C' tadpoles showing retardation of growth by immersion in 0.9 per cent Baker "saponin cholesteride" for an average of one hour daily. A, B, C their untreated controls.

hind limbs, and beginnings of the fore limbs (Stage 19). The treated ones were around Stage 10. Among the controls there were some 4.5 cm. long and some 3.8 cm.; the treated were between 3.3 and 2.8 cm. (Fig. 3).

Tadpoles of Stage 23

In this series, the treated groups were immersed for 1.5 hours each in the same "cholesteride" as the previous cases, also for 15 days. On the eighth day, the controls had well formed jaws and four rows of teeth; the pigment covered only half the abdomen. The treated ones had one row of teeth, and much less abdominal pigmentation. Thin pieces of opercular fold were still visible. On the twentieth day the controls averaged 1.8 cm. × 0.45 against 1.2 × 0.35 cm. of the treated group (Fig. 3).

Summary

It is seen that there is a retardation in development of tadpoles due to "saponin cholesteride." Size as well as stage differences were noted. Microscopic examination of serial sections did not reveal any histologic difference in the treated animals. They were only smaller and retarded in the rate of development.

Action of Non-Hemolytic Saponin-Eosin

According to Noguchi (1906), eosin abolishes, at least partially, the hemolytic power of saponin if the mixture is exposed to the sun. The author kept a 0.3 per cent Merck saponin and 1.5 per cent eosin mixture in the sun for 65 hours. The excess eosin was adsorbed out by charcoal. Trials with this almost cleared solution showed that it was non-hemolytic and capable of retarding growth of onion roots as much as 75 per cent of the water control. Onion roots in similarly treated eosin control were retarded by 25 per cent only.

Discussion and Conclusion

The number of chemicals that can interrupt mitosis is enormous. Yet not all toxic substances do that. For example, the author tried several known poisons and found the following facts: one per cent Lysol kills onion roots but does not prevent mitoses completely. Bromine water diluted 9 times causes cytolysis and disruption of cells too drastic to show mitoses. One per cent pyridine was interesting: while cytoplasm, nuclei and nucleoli of the treated roots were in as perfect form as those of the best control roots, there were no mitotic figures at all. The roots, however, were dead. It warrants further study. Three-tenths of one per cent bile salt mixture kills and softens the roots in two days, yet there were some mitotic figures. It can be said that saponin is more than a general toxic substance, having a specific inhibition on mitosis in addition to its retardation of growth in a general way. Its non-hemolytic "cholesteride" does the same also but in higher concentrations. It is possible that this inhibitory action is the result of disturbances in the water content of the cells. There are several indications in support of this view: the shrinking and curvature of roots, actually becoming brittle, in the saponin and "cholesteride" solutions; the very hygroscopic nature of the compound; and the extensive shrinkage in animal tissues placed in saponin. The role of water in the

growth process is fundamental and a disturbance in water content might be the explanation of retardation in tadpole development and onion root growth. On the other hand, the compound might have an affinity to nuclear constituents. In fact the cytolysis of tissues in saponin, referred to previously (Butros, 1948), indicated a gradual disintegration of the nucleus as revealed by the Feulgen reaction. It is significant to note here that the "saponin cholesteride" had the same cytolytic power on mammalian tissues as saponin.

To conclude, it seems that the whole problem is worth further investigation. As most of this work was done on onion roots, more work should be turned to animal and tissue culture fields. Various other saponins might be tried and might give a milder toxic cholesteride with more inhibitory powers.

Summary

1. Onion root elongation was arrested within two days by 0.1 per cent Merck saponin and 0.3 per cent Baker saponin.

2. No mitotic figures were discerned in these cells; the nuclei of all cells were in the resting stage.

3. Lower concentrations, down to a certain point, reduced the rate of growth of the roots. Below that the very dilute saponins appeared to be growth stimulating.

4. A method of preparing a non-hemolytic suspension of saponin cholesteride is given.

5. The properties of non-hemolytic saponin cholesteride are described. Its toxicity to animals is much less than saponin; also it arrests onion root growth. It also retards the development of tadpoles. Its cytolytic power to mammalian tissues does not seem to have changed.

6. A non-hemolytic preparation of saponin-eosin is found to be partially inhibiting to onion root growth.

7. The possible explanations for this action are discussed.

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